Laboratory methods in genitourinary medicine

Techniques used in clinical immunology

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Introduction

A wide variety of techniques are used in clinical immunology to investigate disorders of the immune system responsible for the clinical features associated with immunodeficiency, allergic or autoimmune states. Some, such as radioimmunoassays and enzyme immunoassays (ELISA), are widely applied techniques for detecting a wide range of proteins such as hormones or specific antibodies and are used in many aspects of laboratory medicine. Their applications are discussed in other contributions to this series and they will only receive passing mention.

The objective of this article is to provide the practising genitourinary physician with a general working explanation of the techniques used to investigate immunological disorders and to provide insight into the precision, reliability and relevance of individual investigations in the assessment of immunological disorders with manifestations in genitourinary medicine. Shortage of space determines that discussion cannot be detailed but further references are included for readers who wish more extensive information.[1-3]

In general, investigation of immune components can be simply divided into the measurement of proteins in blood or body fluids, or the assessment of the cellular compartment especially lymphocyte function. Quantitation of protein components of the immune response includes antibodies but also complement components and mediators or cytokines. All can be measured relatively easily and precisely and normal ranges for age groups and populations are well defined for most components. In contrast, tests of cellular components or function are more difficult to standardise and are technically more complex to perform. Interpretation of results of cellular investigations require reference to normal control material, detailed clinical information and close liaison between requesting clinicians and laboratory based immunologists.

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Measurement of immunoglobulin and other specific serum proteins

Quantitation of total serum immunoglobulin level and serum complement component levels is performed by immunoprecipitation of the required component with a specific antibody. Precipitation at equivalence can be performed in agar gels when visible deposition rings can be measured. Alternatively precipitation in the fluid phase can be measured electronically by nephelometry or turbidimetry. Gel techniques are slow and require large volumes of antibody reagents whereas light scatter techniques are rapid and economical.

Quantitation of immunoglobulin is essential in the assessment of suspected immunodeficiency disorders in patients with recurrent or severe bacterial infections. Immunoglobulin class M G and A levels are low or absent in primary hypogammaglobulinaemia. Such patients present with genital infections only rarely. Respiratory or sinus infections are much more characteristic in patients with these disorders. In patients with recurrent bacterial infections at more than one anatomical site in whom there is a high clinical index of suspicion of underlying antibody deficiency, normal immunoglobulin levels do not rule out antibody deficiency and second line investigations including quantitation of IgG subclasses and functional antibodies may be required.

Immunoglobulin levels are frequently elevated in chronic bacterial or viral infections in the absence of primary hypogammaglobulinaemia. In HIV infections exceptionally high levels of IgG characterise the intermediate stages of HIV infection. IgG levels tend to fall towards the normal range and IgA levels to rise with the establishment of clinical immunodeficiency. Immunoglobulin levels are also elevated in chronic liver disease and a number of multisystem autoimmune diseases such as SLE and rheumatoid arthritis. Their measurement in the latter rarely provides diagnostic information. Individual complement components are consumed during antibody antigen reactions and levels of C3 and C4 frequently fall during active immune complex disease where their quantitation may give some guide to both aetiology and disease activity.

Serum electrophoresis is performed principally to allow a qualitative assessment of serum proteins and

in particular to exclude the presence of monoclonal immunoglobulins. The possibility of a monoclonal immunoglobulin cannot be satisfactorily excluded on the basis of measurement of immunoglobulin class levels in serum alone.

Functional assays of complement activity

Determination of levels of individual complement components do not test the integrity of the whole complement cascade. To exclude individual component deficiency, haemolytic assays (most commonly the CH50 assay) are performed. This estimation quantifies the volume of serum required to lyse 50% of a standard volume of antibody coated red cells and is an *essential* test to exclude individual complement component deficiency. Patients with single component deficiency, particularly of the terminal lytic components C7, C8 and C9 are peculiarly vulnerable to invasive and recurrent neisserial infections and a CH50 examination on *fresh* serum should be performed on patients presenting with gonococcal arthritis or septicaemia.

Detection of total serum IgE and antigen-specific IgE

Antibodies to exogenous non-replicating environmental antigens may result in hypersensitivity or allergy. Antibodies of either IgG or IgE class can be responsible for allergic reactions. In the context of genitourinary medicine IgE antibodies responsible for immediate (type I) reactions are the most relevant. IgE class antibodies are present at low levels even in atopic subjects. Sensitive assay systems such as radioimmunoassay or ELISA techniques are required to quantitate serum levels which are frequently elevated in individuals with an allergic disposition. More valuable in diagnosis is the detection of antigen-specific IgE which can be quantitated directly using a preparation of purified allergen immobilised on a solid phase to which serum antibodies are allowed to bind. After washing of the solid phase any bound IgE antibodies can be detected and quantitated by addition of a radioactively or enzymatically labelled IgE specific antibody. An alternative approach is to perform a skin prick test using a purified antigen and to examine the skin for an immediate "wheal and flare" reaction. Skin prick tests should be the first-line investigation of patients with suspected IgE based reactions. Antigen specific IgE estimations should only be performed when purified skin test antigens are unavailable or skin tests are contraindicated by severe skin disease, previous anaphylaxis or concomitant antihistamine therapy.

Pollens, foods and moulds are all prominent sources of allergens which can trigger IgE based allergic symptoms and in the context of this article it should also be remembered that immediate sen-sitivity to seminal plasma antigens is a rare manifestation of genital allergy in women.

Autoantibody detection

The detection of circulating autoantibodies involves three main methodologies, immunofluorescence, haemagglutination and radio- or enzyme immunoassay. Immunofluorescence is a useful method of rapid screening which obviates the need to purify individual antigens since it allows the visualisation of the tissue distribution of any positive result. Interpretation required skilled UV microscopy of tissue sections. Following their incubation in patient serum, sections on slides are washed and then reincubated with a fluorescein conjugated antihuman immunoglobulin. Antinuclear antibodies, gastric paretal cell, smooth muscle and mitochondial antibodies are amongst the commonly seen autoantibodies routinely detected by this technique.

The haemagglutination technique utilises macroscopic agglutination of red cells coated with purified specific autoantigen following exposure to patients serum to identify any autoantibody present. Haemagglutination is a sensitive and economic technique which is used routinely to detect thyroid autoantibodies and rheumatoid factor.

Autoantibodies which are present in low concentrations require highly sensitive assay systems such as radio- or immunoenzymatic assays for their detection. DNA antibodies, acetylcholine receptor and intrinsic factor antibodies are all quantitated using these more sensitive systems.

Autoantibodies bound in vivo can be detected directly in biopsy samples using immunofluorescence or immunoenzymatic microscopy. Such techniques are very useful in establishing an immunopathological diagnosis and are best performed on fresh snap frozen *unfixed* tissue. A relevant example of its value is in the detection of epidermal autoantibodies which characterise the blistering stain diseases pemphigus and pemphigoid which may be associated with genital lesions. Direct immunofluorescence can also be used to identify immune complex deposition in tissues.

Assessment of lymphocyte function

The increasing frequency and awareness of the cellular immunodeficiency associated with HIV infection has resulted in an increasing number of requests for the assessment of lymphocyte subpopulation numbers and function. Their quantitation gives only a rough guide to the presence of immunodeficiency and whilst, for example, absent or severely reduced CD4 numbers in the absence of intercurrent infection are rarely seen without associated immunodeficiency, normal results do not exclude the latter. In such situations tests of func-

tional competence of the immune system are also required.

The accurate quantitation of lymphocyte populations has become possible because of two factors. Firstly, the increasing availability of monoclonal antibodies specific to individual subpopulations and also the use of flow cytometers which are capable of rapidly and reproducibly enumerating large numbers of positive cells. This reduces the errors inherent in the previously widely used method of visual microscopy in which only small cell numbers were examined and measurement errors correspondingly large.

Accurate cell surface marker analysis requires fresh anticoagulated blood samples which must be received and prepared within 16 hours of venesection. Recent developments allow the staining of cells directly in whole blood preparations reducing further the possibilities of variability inherent in the cell separation techniques previously used. Results may be expressed as percentages of individual populations or as absolute counts derived from the total white cell count and lymphocyte differential. Absolute counts are the preferred method of reporting since percentage counts or CD4/CD8 ratios can be influenced both by a fall in one population or a rise in the other rendering ratio results open to misinterpretation. Results should also be related to normal ranges derived by the local laboratory. Counts may be severely influenced by intercurrent infection, drug therapy or deterioration in samples resulting from delayed receipt.

Lymphocyte surface markers are also used to examine monoclonality of lymphocyte subpopulations in blood and tissue biopsies from patients with suspected leukaemia or lymphoma. Although in most cases of lymphoid malignancy the diagnosis is straightforward on the basis of clinical and convential histopathological criteria, in others the detection and classification of such tumours has been greatly facilitated by the use of monoclonal reagents directed against differentiation antigens expressed on either T or B lymphocyte populations. Expressing cells are usually identified by immunofluorescence and flow cytometry on blood samples or by immunoenzymatic staining on tissue biopsy sections. The latter must be taken unfixed and snap frozen for optimum results.

Tests of T lymphocyte function can be performed either in vivo by skin testing or in vitro by lymphocyte stimulation in tissue culture. Intradermal skin testing using purified challenge antigens to induce delayed-type hypersensitivity reactions appearing at 48 h are a reliable index of retained T cell function. However, positive reactions are critically dependent on prior antigenic exposure. Negative results to a limited panel of antigens, particularly in children, do not necessarily indicate impaired function. In adults, a panel of antigens which includes PPD-tuberculin

(1/1000), candida, tetanus toxoid, and streptokinase streptodornase will elicit a positive response to at least one of these antigens in over 95% of healthy adults. Uncertainty in negative reactors can be examined further by sensitisation to a contact sensitiser such as dinitrochloro-benzene (DNCB) followed by rechallenge 10–14 days later when a delayed reaction should be observed in T lymphocyte competant normal individuals.

In vitro functional tests of T cell immunity are labour intensive and require a high level of technical expertise and are thus only indicated when the clinical suspicion of cellular immunodeficiency is high. Lymphocytes are cultures in the presence of non-specific activators (mitogens) or specific antigens to induce cell division or the release of active mediators (cytokines). Proliferative responses are quantitated by the ability of dividing cells to incorporate the radiolabelled DNA precursor, tritiated thymidine. The ability of T lymphocytes to release active mediators upon antigen challenge is an in vitro correlate of delayed-type hypersensitivity (DTH). Originally, an assay known as the leucocyte migration inhibition test was used to assess DTH. In this assay the ability of the antigens to activate sensitised T lymphocytes was assessed by the limitation of the normal migration of buffy coat leucocytes from a loaded capillary tube. Migration inhibition resulted from the release of cytokine factors from the activated T cells and these assays are being gradually superceded by direct assays for cytokine release in the culture supernatant.

Functional investigations require fresh, sterile anticoagulated blood samples which require lengthy and immediate preparation in the laboratory and are thus only available on a prearranged basis. Standardisation of these techniques is difficult and results are expressed in relation to a simultaneously assayed healthy age matched control sample.

In the assessment of the cellular immunodeficiency associated with HIV infection the additional safety considerations required in the handling of such samples mean that functional investigations are rarely performed. The selectivity and severity of the depletion of the CD4 population associated with progressive HIV associated immunodeficiency is of such a degree that in the clinical assessment of the HIV infected subject the regular quantitation of the absolute CD4 count is a reasonable guide to disease stage in individual patients providing that trends rather than individual count results are observed.

Histocompatability (HLA) testing

Typing of class I and class II major histocompatability antigens is performed on lymphocytes separated from fresh anticoagulated (lithium heparin) blood samples. For class I (A and B locus

alleles) typing whole lymphocyte populations are used whereas for class II (D locus) typing, separated class II bearing B lymphocytes and monocytes populations are prepared from whole lymphocytes by T cell depletion. Cells to be typed are incubated with a panel of different alloantisera specific for individual HLA types. Microcytotoxicity of these cells is observed by microscopy following the addition of lytic complement. Cell killing is only observed if lymphocytes have bound specific antisera allowing the full HLA type to be determined. Although HLA typing is of value in the matching of organ donations, its lack of sensitivity and specificity makes it unreliable for the determination of disease susceptibility in individual cases of HLA associated diseases such as ankylosing spondylitis and Reiter's syndrome. It should not therefore be used for diagnostic purposes in these conditions.

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3 Haeney M. Allergy. In: Gooi HC, Chapel H, eds. *Clinical Immunology: A Practical Approach*. Oxford: Oxford University Press, 1990:221–51.